Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo 2/28/74

BORIC ACID

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SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo

Attached is the report of the investigation of ADIPIC ACID in the developing chicken embryo.

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Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo:

ADIPIC ACID

PROTOCOL:

Adipic acid (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2, 3).

Groups of ten or more eggs were treated under these four conditions at several dose levels until a suitable total number of eggs per level was reached for all levels allowing some to hatch. Groups of adequate size were treated solely with the solvent at corresponding volumes. Untreated controls were also included in each experiment.

After treatment, all the eggs were candled daily and the non-viable embryos were removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. Along with these, histological examinations of major organs (liver, heart, kidney, lung, brain, intestine, gonad, and some endocrine organs) were carried out by taking samples from a representative number of animals from each experimental group.

RESULTS:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of the test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram egg weight, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., the total number of non-viable eggs divided by the total number of treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total number of eggs treated. This includes all the abnormalities observed and also the toxic responses such as edema,

hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia, and neural disorders including ataxia.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as a percentage of the total number of eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Columns 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data in columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

DISCUSSION:

Adipic acid at the higher dose levels was embryotoxic when administered to the embryos under all conditions of the test; the toxicity was significantly (P=0.05) greater than solvent-treated eggs at all dose levels tested at or above 5.0 mg/egg. At the lower dose levels (1.0 and 0.5 mg/egg), the toxicity was not as great as at the higher dose; at 0.5 mg/egg the toxicity was significantly higher in eggs treated via the air cell at 96 hours and via the yolk at 0 hours.

Probit analysis resulted in an LC_{50} of 21.814 mg/egg (air cell at 0 hours, Table 1), an LC_{50} of 35.436 mg/egg (yolk at 0 hours, Table 3) and an LC_{50} of 8.446 mg/egg (yolk at 96 hours, Table 4). Air cell treatment at 96 hours resulted in a line whose slope was not significantly different from zero (Table 2).

Abnormal birds were seen under all of the conditions of the test, although the incidence of birds having a structural abnormality of the head, limbs, viscera, or skeleton was not significantly different from that of the solvent background (P=0.05). Of the 60 control eggs six abnormal birds were found; five of the birds had curled toes and one bird had hip contractures.

AIR CELL AT 0 HOURS: Abnormalities were produced at all tested levels. At 15.0 mg/egg four birds with abnormally curled toes and one with

hip contractures were found. At 10.0 mg/egg, two abnormal birds, one with curled toes and the other with hip contractures, were found. At 5.0 mg/egg two birds had curled toes and two had hip contractures. At 1.0 mg/egg six abnormal birds were produced. All had curled toes. At 0.5 mg/egg four birds with abnormalities were found. Three birds had curled toes and one bird had hip contractures. The solvent-treated eggs produced five birds with curled toes and one bird with hip contractures.

AIR CELL AT 96 HOURS: Abnormalities were found at all of the tested levels except for the 10.0 mg/egg level where no structural abnormalities were found. At 15.0 mg/egg five abnormal birds were found. Four had curled toes and one had hip contractures. At 5.0 mg/egg all three abnormal birds had curled toes. At 1.0 mg/egg three abnormal birds with curled toes and one abnormal bird with hip contractures were produced. At 0.5 mg/egg five birds with abnormalities were found. One bird had hip contractures and four had curled toes. The solvent-treated eggs produced four birds with curled toes and two birds with hip contractures.

YOLK SAC AT 0 HOURS: Abnormalities were seen at all tested dosages. At 15.0 mg/egg two abnormal birds with curled toes were produced. At 10.0 mg/egg the two abnormal birds found had hip contractures. At 5.0 mg/egg three abnormal birds were found with one of the following: hip contractures; abdominal celosomia; and curled toes. At 1.0 mg/egg all four abnormal birds found had curled toes. At the 0.5 mg/egg level a total of four abnormal birds were produced. Two had curled toes and two had hip contractures. In the solvent-treated eggs there were two abnormalities; one bird with hip contracture and one bird with curled toes.

YOLK SAC AT 96 HOURS: At 15.0 mg/egg four abnormal birds with hip contractures and one abnormal bird with curled toes were found. At 10.0 mg/egg one abnormal bird was produced. It had curled toes. At 5.0 mg/egg four abnormal birds were seen. One bird had curled toes and three birds had hip contractures. At 1.0 mg/egg two birds, one with hip contractures and the other with curled toes, were found. At 0.5 mg/egg four abnormal birds with curled toes were produced. Of the solvent-treated eggs all four of the abnormal birds found had curled toes.

Microscopical examination of the paraffin embedded and H&E stained sections revealed no consistent histological changes in any of the organs observed. Although occasional hemorrhage, vacuolization, or fatty infiltration in the liver were seen, neither of these changes correlated with the administered dose nor the varieties of the external abnormalities.

Judging from all of the test results, it is concluded that adipic acid is not teratogenic to the chicken embryo even at relatively high dosages. Most of the abnormalities found in this test were non-specific; i.e., they were also found in the solvent-treated and untreated controls.

1. Adipic Acid, Food processing quality, E. I. DuPont, De NeMours & Co., Wilmington, Delaware

2. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M.J., Mutchler, M.K. and Fitzhugh, O.G. Toxicol. Appl. Pharmacol. 5:760-770, 1963

3. Verrett, M.J., Marliac, J.-P. and McLaughlin, J., Jr. JAOAC 47: 1002-1006, 1964

4. Finney, D.J. Probit Analysis, 2nd ed., Cambridge Press, Cambridge, Appendix I, 1964

Table 1

Adipic Acid

Air Cell at 0 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
15.0	300	72	63.88*	6.94	6.94
10.0	200	72	55.55*	4.16	2.77
5.0	100	76	47.36*	7.89	5.26
1.0	20	75	38.66	8.00	8.00
0.5	10	74	40.54	6.75	5.40
Water		51	23.52	11.76	11.76
Control		60	13.33	10.00	10.00

LC_{30} 3.621 mg/egg (72.428 mg/kg)

LC_{50} 21.814 mg/egg (436.289 mg/kg)

LC_{90} 1756.623 mg/egg (35132.460 mg/kg)

*Significantly different from solvent $P \leq 0.05$

Table 2

Adipic Acid

Air Cell at 96 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
15.0	300	72	66.66*	8.33	6.94
10.0	200	72	58.33*	2.77	.00
5.0	100	67	43.28*	5.97	4.47
1.0	20	73	50.68*	5.47	5.47
0.5	10	71	47.88*	7.04	7.04
Water		54	22.22	11.11	11.11
Control		60	13.33	10.00	10.00

P (calculated) $<$ P(0.05)

*Significantly different from solvent P $\leq$ 0.05

Table 3

Adipic Acid

Yolk Sac at 0 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
15.0	300	71	63.38*	2.81	2.81
10.0	200	70	52.85*	7.14	2.85
5.0	100	74	48.64*	4.05	4.05
1.0	20	75	44.00	5.33	5.33
0.5	10	75	45.33*	5.33	5.33
Water		51	25.49	5.88	3.92
Control		60	13.33	10.00	10.00

LC_{30} 1.547 mg/egg (30.957 mg/kg)

LC_{50} 35.436 mg/egg (708.738 mg/kg)

LC_{90} 74564.900 mg/egg (1491298.000 mg/kg)

*Significantly different from solvent $P \leq 0.05$

Table 4

Adipic Acid

Yolk Sac at 96 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
15.0	300	70	71.42*	7.14	7.14
10.0	200	72	55.55*	1.38	1.38
5.0	100	75	46.66*	5.33	5.33
1.0	20	61	34.42	3.27	3.27
0.5	10	75	36.00	5.33	5.33
Water		53	18.86	7.54	7.54
Control		60	13.33	10.00	10.00

LC_{30} 1.584 mg/egg (31.696 mg/kg)

LC_{50} 8.446 mg/egg (168.926 mg/kg)

LC_{90} 45.015 mg/egg (900.304 mg/kg)

*Significantly different from solvent $P \leq 0.05$